UNIVERSITI PUTRA MALAYSIA

EFFECTS OF HARVESTING AGE, DRYING, TEA FORM, STORAGE AND INFUSION TIME ON PHYSICOCHEMICAL CHARACTERISTICS OF Clinacanthus nutans (Burm. F) LINDAU

MUNIRAH MOHAMAD

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By

MUNIRAH MOHAMAD

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

July 2017

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DEDICATION

Dedicated to my beloved parents, husband, children, siblings, in laws, supervisors and fellow friends for their endless love, support, understandings, sacrifices, motivation, advice and encouragement.

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in Fulfillment of the requirement for the Degree of Doctor of Philosophy

EFFECTS OF HARVESTING AGE, DRYING, TEA FORM, STORAGE AND INFUSION TIME ON PHYSICOCHEMICAL CHARACTERISTICS OF *Clinacanthus nutans* (Burm. F) LINDAU

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July 2017

Chairman : Associate Professor Siti Hajar Ahmad, PhD
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One of the main herbs well known for its medicinal value is the Sabah snake grass or 'Belalai gajah' (*Clinacanthus nutans*). The primary chemical constituents of the leaves are schaftoside, vitexin, isovitexin, orientin and isoorientin, and antiviral activity is shown by two glycoglycerolipids. Despite the importance of *C. nutans*, complete information with respect to commercial production and postharvest handling, such as plant vegetative stage (young and mature), drying method (oven, sun, solar and air), storage form (powdered and shredded) and storage duration (0, 4, 8 and 12 weeks) of the herb in the local herbal industry is still lacking. Thus, the objective of this study was to determine the optimum postharvest handling processes that could retain the physicochemical quality of *C. nutans*. Results of Experiment 1 showed that moisture content of all drying method were not more than 15%. In addition, it indicated that colour (L*, C* and h^o), chlorophyll content, phytochemical properties and flavonoid compounds (orientin, isoorientin, vitexin, isovitexin and schaftoside) were higher in the young vegetative stage than mature vegetative stage. All the physicochemical qualities were highest when the samples were oven dried compared to the sun, air and solar dried. Furthermore, microbial contamination was not more than the permissible limit by World Health Organisation (WHO). A further study in Experiment 2 showed that the powdered sample resulted in highest phytochemical properties and flavonoid compounds compared to the shredded form. In general, 3 months of storage did not affect the *C. nutans* quality. Experiment 3 focused on comparing the effect of infusion time of *C. nutans* tea at young vegetative stage, oven dried and in powdered form (from Experiment 1 and 2) with *C. nutans* teas available in the local market. The colour, phytochemicals properties and flavonoid compounds infused at 2 and 5 min were significantly different. Increased infusion time at 5 min yield more phytochemical properties and flavonoid compounds than those infused at 2 min. Tea from different producers showed great distinction in their physicochemical and safety level. It was observed that the quality

properties of the teas examined were considerably at second place in comparison with those of the *C. nutans* teas available in the local market. Fortunately, microbial and heavy metal contaminations for all producers were not more than the permissible limit by WHO. As conclusion young-oven dried and powdered form contained highest value in physicochemical characteristics during postharvest handling of *Clinacanthus nutans.*

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN PERINGKAT PENUAIAN, PENGERINGAN, BENTUK PENYIMPANAN TEH, MASA PENYIMPANAN DAN MASA RENDAMAN TERHADAP FIZIKOKIMIA *Clinacanthus nutans* (Burm. F) LINDAU

Oleh

MUNIRAH MOHAMAD

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Pengerusi : Profesor Madya Siti Hajar Ahmad, PhD
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Salah satu herba utama yang semakin terkenal dengan nilai perubatannya adalah Sabah snake grass atau Belalai gajah (*Clinacanthus Nutans*). Kandungan kimia utama herba ini adalah schaftoside, vitexin, isovitexin, orientin dan isoorientin serta aktiviti antivirus ditunjukkan oleh dua glycoglycerolipids. Meskipun pelbagai kebaikan *C. nutans* dilaporkan, maklumat lengkap berkenaan dengan pengeluaran komersial dan pengendalian lepas tuai, seperti masa tuaian (muda dan tua), kaedah pengeringan (ketuhar, matahari, udara dan solar), bentuk simpanan (serbuk dan dicincang) dan tempoh penyimpanan (0, 4, 8 dan 12 minggu) herba ini dalam industri masih kurang. Oleh itu, objektif kajian ini adalah untuk menentukan proses pengendalian lepas tuai optimum yang boleh mengekalkan kualiti fizikokimia *C. nutans*. Keputusan Eksperimen 1 menunjukkan bahawa kandungan air di dalam sampel tidak melebihi daripada 15%. Di samping itu, warna (L *, C * dan h^o), kandungan klorofil, kandungan fitokimia dan sebatian flavonoid (vitexin, orientin, isovitexin, isoorientin dan schaftoside) adalah lebih tinggi pada peringkat vegetatif muda daripada peringkat vegetatif matang. Semua kualiti fizikokimia adalah tertinggi kandungannya apabila sampel dikeringkan di dalam ketuhar berbanding pengeringan dengan menggunakan matahari, udara dan solar. Pencemaran mikrob tidak melebihi daripada had yang dibenarkan oleh World Health Organisation (WHO). Satu kajian lanjut dalam Eksperimen 2 menunjukkan bahawa sifat fitokimia yang tertinggi didapati pada sampel serbuk dan sebatian flavonoid berbanding sampel yang dicincang. Secara umum, 3 bulan penyimpanan tidak menjejaskan kualiti *C. nutans*. Eksperimen 3 tertumpu kepada membandingkan kesan masa rendaman teh *C. nutans* yang dituai pada peringkat vegetatif muda, dikeringkan dengan ketuhar dan dalam bentuk serbuk (dari Experiment 1 dan 2) dengan teh *C. nutans* yang terdapat di pasaran tempatan. Warna, kandungan fitokimia dan sebatian flavonoid direndam pada 2 dan 5 min berbeza secara ketara. Peningkatan masa infusi

pada 5 min menunjukkan kandungan fitokimia dan sebatian flavonoid lebih tinggi daripada yang diinfusi pada 2 min. Teh dari pengeluar yang berbeza menunjukkan perbezaan yang besar dalam kandungan fizikokimia dan tahap keselamatan. Ia menunjukkan bahawa kandungan kualiti teh yang diuji berada di tempat kedua berbanding dengan teh *C. nutans* yang terdapat di pasaran tempatan. Pencemaran mikrob dan logam berat untuk semua pengeluar tidak melebihi daripada had yang dibenarkan oleh WHO. Kesimpulannya, muda-pengeringan ketuhar serta dalam bentuk serbuk menunjukkan kandungan fisikokimia tertinggi semasa pengendalian lepas tuai *Clinacanthus nutans.*

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My sincere gratitude to all the staff, who have helped me to achieve this study. I'm also thankful to my colleagues for their friendship and encouragement.

I certify that a Thesis Examination Committee has met on 7 July 2017 to conduct the final examination of Munirah binti Mohamad on her thesis entitled "Effects of Harvesting Age, Drying, Tea Form, Storage and Infusion Time on Physicochemical Characteristics of *Clinacanthus nutans* (Burm. F) Lindau" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		iii
ACKNOWLEDGEMENTS		v
APPROVAL		vi
DECLARATION		viii
LIST OF TABLES		xiii
LIST OF FIGURES		xvi
LIST OF ABBREVIATIONS		xix
LIST OF APPENDICES		xxi
CHAPTER		
1	**GENERAL INTRODUCTION**	1
2	**LITERATURE REVIEW**	4
2.1	*Clinacanthus nutans*	4
2.1.1	Cultivation	4
2.1.2	Importance of *C. nutans*	6
2.1.2	Biological activities	6
2.2	Plant development stages	8
2.3	Drying methods	9
2.3.1	Oven drying	9
2.3.2	Sun drying	10
2.3.3	Solar drying	11
2.3.4	Air drying	11
2.4	Storage duration	12
2.5	Tea	13
2.5.1	Leaf form	13
2.5.2	Infusion	13
2.6	Postharvest quality characteristics	14
2.6.1	Moisture loss	14
2.6.2	Chlorophyll and colour	15
2.6.3	Phytochemicals and antioxidant activity	16
2.6.4	Flavonoids compounds	18
2.6.5	Microbial contamination	18
2.6.6	Heavy metal contents	19
3	**PHYSICOCHEMICAL CHARACTERISTICS AND MICROBIAL CONTAMINATION OF *Clinacanthus nutans* AS AFFECTED BY PLANT VEGETATIVE STAGES AND DRYING METHODS**	20
3.1	Introduction	20
3.2	Materials and methods	21
3.2.1	Plant materials	21
3.2.2	Drying processes	22

3.2.2.1 Oven drying 22
3.2.2.2 Solar drying 23
3.2.2.3 Air drying 23
3.2.2.1 Sun drying 23
3.2.3 Determination of physicochemical characteristics 24
3.2.3.1 Moisture content 24
3.2.3.2 Chlorophyll content 24
3.2.3.3 Leaf colour 24
3.2.4 Determination of phytochemical property 25
3.2.4.1 Sample extraction 25
3.2.4.2 Total phenolic content 26
3.2.4.3 Total flavonoid content 26
3.2.4.4 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay 26
3.2.4.5 Ferric reducing antioxidant power (FRAP) assay 27
3.2.5 Identification and quantification of flavonoid compound contents in *C. nutans* by ultra fast liquid chromatography (UFLC) 27
3.2.5.1 Standard, reagents and samples 27
3.2.5.2 Solid phase extraction (SPE) 27
3.2.5.3 Preparation of standard solution 27
3.2.5.4 UFLC separation parameters 28
3.2.6 Determination of microbial contamination of *C. nutans* 29
3.2.6.1 Preparation of media 29
3.2.6.2 Total plate count 29
3.2.7 Experimental design and data analysis 29
3.3 Results and discussion 30
3.3.1 Moisture and chlorophyll content 30
3.3.2 Colour attributes 33
3.3.3 Correlations between physical and chemical attributes 37
3.3.4 Phytochemical content and activity 38
3.3.5 Flavonoid compounds 44
3.3.6 Correlations between phytochemicals and flavonoid compounds attributes 48
3.3.7 Microbial contamination 49
3.4 Conclusion 50

4 PHYSICOCHEMICAL CHARACTERISTICS AND MICROBIAL CONTAMINATION OF *Clinacanthus nutans* IN SHREDDED AND POWDERED FORM DURING STORAGE 51
4.1 Introduction 51
4.2 Materials and methods 52
4.2.1 Plant materials 52
4.2.2 Drying and storage treatments 53
4.2.3 Determination of physicochemical characteristics 53
4.2.3.1 Moisture content 53
4.2.3.2 Chlorophyll content 53
4.2.3.3 Leaf colour 54

4.2.4 Determination of phytochemical property 54
4.2.5 Identification and quantification of flavonoid compound contents in *C. nutans* by ultra fast liquid chromatography (UFLC) 54
4.2.6 Determination of microbial contamination of *C. nutans* 54
4.2.7 Experimental design and statistical analysis 54
4.3 Results and discussion 55
4.3.1 Moisture loss and chlorophyll contents 55
4.3.2 Colour 58
4.3.3 Phytochemicals property 62
4.3.4 Identification and quantification of flavonoid compounds 64
4.3.3 Microbial contamination 66
4.4 Conclusions 69

5 DIFFERENT INFUSION TIMES OF *Clinacanthus nutans* TEA AFFECT COLOUR, FLAVONOID COMPOUNDS, MICROBIAL CONTAMINATION AND HEAVY METAL CONTENTS 70

5.1 Introduction 70
5.2 Materials and methods 71
5.2.1 Plant materials 71
5.2.2 Tea infusion preparation 72
5.2.3 Determination of physicochemical characteristics 72
5.2.3.1 Chlorophyll content 72
5.2.3.2 Leaf colour 72
5.2.4 Determination of phytochemical contents and activities 72
5.2.5 Identification and quantification of flavonoid compound contents in *C. nutans* by ultra fast liquid chromatography (UFLC) 72
5.2.6 Determination of microbial contamination of *C.nutans* 72
5.2.4 Determination of heavy metals content 73
5.2.3 Experimental design and statistical analysis 73
5.3 Results and discussion 73
5.3.1 Chlorophyll content and colour attributes 73
5.3.2 Phytochemical property 80
5.3.3 Flavonoid compounds 83
5.3.4 Microbial contaminations 88
5.3.5 Heavy metals contaminations 90
5.4 Conclusions 92

6 GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH 93

REFERENCES 95
APPENDICES 127
BIODATA OF THE STUDENT 137
LIST OF PUBICATIONS 138

LIST OF TABLES

Table		Page
3.1	Gradient composition of mobile phase for flavonoid separation by UFLC	29
3.2	Main and interaction effects of plant vegetative stages (young and mature) and drying methods (oven, solar, air and sun) on moisture loss and chlorophyll contents of *Clinacanthus nutans*.	31
3.3	Main and interaction effects of plant vegetative stages (young and mature) and drying methods (oven, solar, air and sun) on hue (h°), lightness (L*), chroma (C*) and colour changes (ΔE) of *Clinacanthus nutans*.	34
3.4	Pearson's correlation coefficients (r) between physical attributes measured in dried *Clinacanthus nutans* obtained at young and mature vegetative stages.	39
3.5	Main and interaction effects of plant vegetative stages (young and mature) and drying methods (oven, solar, air and sun) on total phenolic content (TPC), total flavonoid content (TFC), 2,2, diphenyl-1-phenyldrazyl (DPPH) and ferric reducing antioxidant plasma (FRAP) of *Clinacanthus nutans.*	40
3.6	Main and interaction effects of plant vegetative stages and drying methods on vitexin orientin, schaftoside and isovitexin content of *Clinacanthus nutans*.	46
3.7	Correlation coefficients (r) between physical and chemical attributes measured in dried *Clinacanthus nutans* obtained at young and mature vegetative stages.	50
3.8	Main and interaction effects of plant vegetative stages (young and mature) and drying methods (oven, solar, air and sun) on microbial contamination of *Clinacanthus nutans.*	52
4.1	The main and interaction effects between sample form (shredded and powdered) and storage duration (week 0, 4, 8 and 12) on moisture loss and chlorophyll content of C*linacanthus nutans* stored at ambient temperature.	58

4.2 Main and interaction effects between sample form (shredded and powdered) and storage duration (week 0, 4, 8 and 12) on chlorophyll a, chlorophyll b and chlorophyll a/b of C*linacanthus nutans* stored at ambient temperature. 59

4.3 Main and interaction effects between sample form (shredded and powdered) and storage week (0, 4, 8 and 12) on hue (h°), lightness (L*), chroma (C*), and colour changes (ΔE) of C*linacanthus nutans* stored at ambient temperature. 61

4.4 Main and interaction effects between sample form (shredded and powdered) and storage week (week 0, 4, 8 and 12) on total phenolic content (TPC), total flavonoid content (TFC), 2,2, diphenyl-1-phenyldrazyl (DPPH) and ferric reducing ability of plasma (FRAP) of *Clinacanthus nutans* stored at ambient temperature. 65

4.5 Main and interaction effects between sample form (shredded and powdered) and storage week (0, 4, 8 and 12) on vitexin orientin, schaftoside and isovitexin contents of *Clinacanthus nutans* stored at ambient temperature. 69

4.6 Main and interaction effects between sample form (shredded and powdered) and storage week (week 0, 4, 8 and 12) on bacteria and fungi contamination of *Clinacanthus nutans* stored at ambient temperature. 70

5.1 Main and interaction effects of infusion time (2 and 5 min) and types of *Clinacanthus nutans* tea (Products 1 to 6) on contents of chlorophyll a, chlorophyll b and total chlorophyll. 78

5.2 Main and interaction effects of infusion time (2 and 5 min) and types of *Clinacanthus nutans* tea (Products 1 to 6) on contents of hue (h°), lightness (L*) and chroma (C*). 79

5.3 Main and interaction effects of infusion time (2 and 5 min) and types of *Clinacanthus nutans* tea (control and products 2 to 6) on total phenolic content (TPC), total flavonoid content (TFC), 2,2, diphenyl-1-phenyldrazyl (DPPH) and ferric reducing antioxidant plasma (FRAP) of *Clinacanthus nutans***.** 84

5.4 Main and interaction effects of infusion time (2 and 5 min) and types of *Clinacanthus nutans* tea (Products 1 to 6) on vitexin and isoorientin content of *Clinacanthus nutans*. 88

5.5 Main and interaction effects of infusion times (2 and 5 min) and types of *Clinacanthus nutans* tea (Products 1 to 6) on orientin, schaftoside and isovitexin content of *Clinacanthus nutans*. 89

5.6 Main and interaction effects of infusion time (2 and 5 min) and types of *Clinacanthus nutans* tea (control and Products 2 to 6) on bacterial and fungal contaminations of *Clinacanthus nutans*. 93

5.7 Cadmium (Cd), mercury (Hg), arsenic (Ar) and plumbum (Pb) contaminations of different types of *Clinacanthus nutans* tea products (Products 1 to 6). 95

LIST OF FIGURES

Figure		Page
2.1	Vegetative characteristics of *C. nutans* branches (A), stem attached by petioles with leaves (B), simple lanceolate leaves (C) and opposite leaf arrangement (D).	5
3.1	The 1976 Commission Internationale de l'Eclairage (CIE Lab) and Lightness, chromaticity and hue colour systems (Precise Colour Communication), Minolta, (2007).	26
3.2	Effect of drying methods (oven, solar, air and sun) on chlorophyll content of *Clinacanthus nutans* plants at young and mature vegetative stages.	32
3.3	Colour variables obtained from different drying methods (oven, solar, air and sun) in respective of young and mature vegetative stages of *Clinacanthus nutans*.	35
3.4	Effect of drying methods (oven, solar, air and sun) on total phenolic content of *Clinacanthus nutans* plants at young and mature vegetative stages.	40
3.5	Effect of drying methods (oven, solar, air and sun) on total flavonoid content of *Clinacanthus nutans* plants at young and mature vegetative stages.	41
3.6	Effect of drying methods (oven, solar, air and sun) on 2,2, diphenyl-1-phenyldrazyl of *Clinacanthus nutans* plants at young and mature vegetative stages.	44
3.7	Ultra fast liquid chromatography profile of young *Clinacanthus* dried using oven (A), solar (B), air (C) sun (D) and mature vegetative stages (E), orientin (1), schaftoside (2), vitexin (3) and isovitexin (4) peaks.	46
3.8	Effect of drying methods on (A) schaftoside and (B) isovitexin of *Clinacanthus nutans* plants at young and mature vegetative stages.	47
4.1	Relationship between storage week and chlorophyll content of *Clinacanthus nutans* in shredded and powdered forms stored at ambient temperature. Plant samples contained leaf and branch tissues. The solid line indicates a significant regression	58

at P ≤ 0.05, n=5.

4.2 Shredded (A) and powdered (B) *Clinacanthus nutans* during storage weeks 0, 4, 8 and 12. Plant samples contained both leaf and branch tissues harvested from a 1.5 month one-time ratooned plant. At harvest, all 50-cm tissues of aerial branch and leaf were obtained. 62

4.3 Relationship between storage week and chroma value of *Clinacanthus nutans* in shredded and powdered forms stored at ambient temperature. Plant samples contained leaf and branch tissues. The solid line indicates a significant regression at P ≤ 0.05, n = 5. 63

4.4 UFLC profile of different sample form of oven dried *C. nutans* and storage weeks 0, 4, 8, 12. (A) powdered sample;(B) shredded sample. Peaks (1) orientin; (2) shaftoside (3) vitexin (4) isovitexin. 68

4.5 Relationship between storage week and bacterial count of shredded and powdered *Clinacanthus nutans* branch and leaf tissues stored at ambient temperature. The solid line indicates a significant regression at P ≤ 0.05, n = 5. 71

5.1 *Clincanthus nutans* tea products consisting of the Product 1 and five commercial products (Products 2 to 6). The Product 1 as the control comprised of young plant-parts, oven-dried and in powdered form (selected treatment from Chapter 3 and 4). Product 2 contained only dried-leaves while Products 3 to 6 contained dried branches and leaves of the plant. 77

5.2 Effect of infusion time (2 and 5 min) on hue (h^o) of *Clinacanthus nutans* tea (Products 1 to 6). 80

5.3 Effect of infusion time (2 and 5 min) on (A) chroma and (B) lightness of *Clinacanthus nutans* tea (Products 1 to 6). 81

5.4 Effect of infusion time (2 and 5 min) on total phenolic content of *Clinacanthus nutans* tea (Products 1 to 6). 84

5.5 Effect of infusion time (2 and 5 min) on (A) total flavonoid content and (B) ferric reducing antioxidant plasma of *Clinacanthus nutans* tea (Products 1 to 6). 85

5.6 Effect of infusion time (2 and 5 min) on (A) total flavonoid content and (B) ferric reducing antioxidant plasma of *Clinacanthus nutans* tea (Products 1 to 6). 86

5.7	Ultra Fast Liquid Chromatography profile of Product 1 (P1) as control, Product 2 (P2), Product 3 (P3), Product 4 (P4), Product 5 (P5) and Product 6 (P6) of *Clinacanthus nutans* from Malaysian market. Flavanoid compounds elucidated were orientin (1), schaftoside (2), isoorientin (3), vitexin (4) and isovitexin (5).	87
5.8	Effect of infusion time (2 and 5 min) on (A) orientin and (B) schaftoside content of *Clinacanthus nutans* tea (Products 1 to 6).	90
5.9	Effect of infusion time (2 and 5 min) on schaftoside content of *Clinacanthus nutans* tea (Products 1 to 6).	90
5.10	Effect of infusion time (2 and 5 min) on isovitexin of *Clinacanthus nutans* tea (Products 1 to 6).	91

LIST OF ABBREVIATIONS

%	Percent
µl	Microliter
Al	Aluminium
$AlCl_3$	Aluminum chloride
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
As	Arsenic
C^*	Chroma
Cd	Cadmium
CFU g^{-1}	Colony forming units per gram of sample
CH_2O_2	Formic acid
CH_3CN	Acetonitrile
CH_3OH-d4	Methanol-d4
Cm	Centimeter
Co	Cobalt
Cr	Chromium
CRD	Completely randomized design
Cu	Copper
D	Drying
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	Dry weight
FDA	Food and Drug Administration
$FeCl_3.6H_2O$	Iron (III) Chloride Hexahydrate
FRAP	ferric reducing antioxidant power
G	Gram
H	Hour
h°	Hue angle
HCl	Hydrochloric acid
Hg	Mercury
HPLC	High Performance Liquid Chromatography
ICP-OES	Inductively coupled plasma optical emission spectrometry
$K_2S_2O_8$	Potassium persulfate
Kg	Kilogram
KH_2PO_4	Potassium dihydrogen phosphate
kPa	Kilopascal
L	Liter
L^*	Lightness
LSD	Least significant difference
M	Meter
M	Molar
m^2	Meter square
mbar	Millibars
MeOH	Methanol
Mg	Milligram
mg g^{-1}	Milligram per gram

mg kg^{-1}		Milligram per kilogram
mg L^{-1}		Milligram per liter
mg mL^{-1}		Milligram per mililiter
mg N/ha		Milligram nitrogen per hectar
Min		Minutes
mL		Mililiter
mL L^{-1}		Mililiter per liter
mL/min		Mililiter per minutes
ml-micro		Milliliter micro
Mm		Millimeter
mM		Millimolar
MSA		Mannitol Salt Agar
NA		Nutrient agar
NaOD		Sodium deuterium oxide
NaOH		Sodium hydroxide
ND		Not detected
Ni		Nickel
Ns		No significant
ºC		Degree celcius
Pb		Lead
Ppm		Parts per million
QE		Quercetin equivalent
R^2		R square
S		Storage
SE		Standard Error
TE/g		Milligram Trolox equivalent
TFC		Total flavonoid content
TNTC	:	Too numerous to count
ton/ha		Ton / hectare
TPC		Total phenolic content
TPTZ		6-tripyridyl-striazine
TPU		Taman Pertanian Universiti
TSP		3-(trimethylsilyl) propionic-2,2,3,3-d4 acid sodium salt
UPM		Universiti Putra Malaysia
UV/VIS		Ultraviolet–visible
WHO		World Health Organization
Zn		Zinc
Mm		Micrometer

LIST OF APPENDICES

Appendix		Page
3.1	The photograph of the solar dryer at Green Energy Technology Innovation Park, UKM Malaysia consisted of a blower, auxiliary heater, double-pass solar collector and a drying chamber.	185
3.2	The photograph of drying house at Taman Pertanian Universiti, UPM with high quality transparent plastic for roof.	185
3.3	Analysis of variance of plant vegetative stages (young and mature) and drying methods (oven, solar, air and sun) on moisture loss and chlorophyll contents of *Clinacanthus nutans*.	186
3.4	Analysis of variance of plant vegetative stages (young and mature) and drying methods (oven, solar, air and sun) on hue (h°), lightness (L*), chroma (C*) and colour changes (ΔE) of *Clinacanthus nutans*.	186
3.5	Analysis of variance of plant vegetative stages (young and mature) and drying methods (oven, solar, air and sun) on total phenolic content (TPC), total flavonoid content (TFC), 2,2, diphenyl-1-phenyldrazyl (DPPH) and ferric reducing antioxidant plasma (FRAP) of *Clinacanthus nutans.*	187
3.6	Analysis of variance of plant vegetative stages and drying methods on vitexin orientin, schaftoside and isovitexin content of *Clinacanthus nutans*.	187
3.7	Analysis of variance of of plant vegetative stages (young and mature) and drying methods (oven, solar, air and sun) on microbial contamination of *Clinacanthus nutans.*	188
4.1	Analysis of variance ofeffects between sample form (shredded and powdered) and storage duration (week 0, 4, 8 and 12) on moisture loss and chlorophyll content of C*linacanthus nutans* stored at ambient temperature.	189
4.2	Analysis of variance ofeffects between sample form (shredded and powdered) and storage duration (week 0, 4, 8 and 12) on chlorophyll a, chlorophyll b and chlorophyll a/b of C*linacanthus nutans* stored at ambient temperature.	189

4.3	Analysis of variance of effects between sample form (shredded and powdered) and storage week (0, 4, 8 and 12) onhue (h°), lightness (L*), chroma (C*), and colour changes (ΔE) of C*linacanthus nutans* stored at ambient temperature.	190
4.4	Analysis of variance of effects between sample form (shredded and powdered) and storage week (week 0, 4, 8 and 12) on total phenolic content (TPC), total flavonoid content (TFC), 2,2, diphenyl-1-phenyldrazyl (DPPH) and ferric reducing antioxidant plasma (FRAP) of *Clinacanthus nutans* stored at ambient temperature.	190
4.5	Analysis of variance of effects between sample form (shredded and powdered) and storage week (0, 4, 8 and 12) on vitexin orientin, schaftoside and isovitexin contents of *Clinacanthus nutans* stored at ambient temperature.	191
4.6	Analysis of variance of effects between sample form (shredded and powdered) and storage week (week 0, 4, 8 and 12) on bacteria and fungi contamination of *Clinacanthus nutans* stored at ambient temperature.	191
5.1	Analysis of variance of effects of infusion time (2 and 5 min) and types of *Clinacanthus nutans* tea (Products 1 to 6) on contents of chlorophyll a, chlorophyll b and total chlorophyll.	192
5.2	Analysis of variance of effects of infusion time (2 and 5 min) and types of *Clinacanthus nutans* tea (Products 1 to 6) on contents of hue (h°), lightness (L*) and chroma (C*).	192
5.3	Analysis of variance of effects of infusion time (2 and 5 min) and types of *Clinacanthus nutans* tea (control and products 2 to 6) on total phenolic content (TPC), total flavonoid content (TFC), 2,2, diphenyl-1-phenyldrazyl (DPPH) and ferric reducing ability of plasma (FRAP).	193
5.4	Analysis of variance of effects of infusion time (2 and 5 min) and types of *Clinacanthus nutans* tea (Products 1 to 6) on vitexin and isoorientin content.	193
5.5	Analysis of variance of effects of infusion time (2 and 5 min) and types of *Clinacanthus nutans* tea (Products 1 to 6) on orientin, schaftoside and isovitexin content.	194
5.6	Analysis of variance of effects of infusion time (2 and 5 min) and types of *Clinacanthus nutans* tea (control and	194

products 2 to 6) on bacterial and fungal contaminations.

5.7 Analysis of variance of Cadmium (Cd), mercury (Hg), arsenic (Ar) and plumbum (Pb) contaminations of different types of *Clinacanthus nutans* tea products (Products 1 to 6). 195

CHAPTER 1

GENERAL INTRODUCTION

In recent years, it is estimated that 70-80% of people worldwide rely mainly on medicinal herbs to meet their primary healthcare needs. The global demand for herbal medicine is not only large but also a growing one (Khan et al., 2006). According to a report by the United Nation (2000), the world market for medicinal herbs is estimated around US$60 billion annually. One local herb, the Sabah snake grass (*Clinacanthus nutans*) is increasingly in demand and is listed among 18 important herbs under the National Key Economic Area (NKEA) due to health awareness, and potential as an alternative medical treatment (MOA, 2012). *C. nutans* belongs to the family of Acanthaceae that is native to tropical Asia and found growing in Malaysia, Vietnam, Indonesia and China (Ayudhya et al., 2001). This herbal plant species is usually consumed as an herbal tea and widely used by cancer patients due to its high bioactive compounds and phytochemicals content. It has been traditionally used in Thailand as an anti-snake venom treatment. Clinical trials of *C. nutans* extracts for ailments such as insect bite and virus lesions (herpes simplex and varicella-zoster) also have been reported (Yoosook et al., 1999). Also, *C. nutans* is commonly consumed in the form of herbal tea, which is effective for treating diabetes mellitus, fever, dysuria and diarrhea (Uawonggul et al., 2011). The primary chemical constituents of the leaves are vitexin, isovitexin, orientin and isooreintin, schaftoside and antiviral activity is shown by two glycoglycerolipids (Sakdarat et al., 2009).

In Malaysia, there is a high demand for fresh leaves of *C. nutans.* It is sold at RM 40/kg and extracts are sold at RM 89/bottle (1L) (Fong, 2015). Despite the importance of *C. nutans*, scientific research shows that this medicinal herb is often of variable quality. Many studies on other herbs have shown that different plant age and maturity or stage of development would lead to differences in quality. For example, the yield, oil and bioactive compound contents of fresh thyme (*Thymus vulgaris* L.) are affected by harvesting stage (Badi et al., 2004). In another example, Esmelindro et al. (2004) reported that young *Ilex paraguariensis* produced higher methylxanthines. In dried herbs, contents of total lactones were maximum at the pre-flowering stage and lowest at the flowering stage (Singh et al., 2011). Maximum oil production of Indian basil (*Ocimum basilicum* L.) occurred around 40 days after transplanting; thus it was suggested that the herb be harvested at around that time (Singh et al., 2010). Thus, a correct harvesting stage is an important agronomic factor that affects quality characteristics of herbs.

Unfortunately, good drying methods for sustainable *C. nutans* cultivation have not been well studied and documented. Drying treatments are important to reduce the amounts of moisture in the herbs which would enable extension of shelf life of the herbs. Drying also alters other physical, biological and chemical properties of foods (Demirhan and Özbek, 2010). Drying of herbs inhibits microbial growth and forestalls certain biochemical changes, but at the same time, it can give rise to other

alterations that affect herb quality. As reported by Orphanides et al. (2013) sun drying of spearmint produced high total phenolic content (TPC) and potent antioxidant activity compared to oven and solar drying. Thus, different drying methods would give different effects on the quality and bioactive compounds in the plant itself. For sustainable commercial cultivation of this valuable herb, suitable plant age and drying methods that maintain quality and reduce postharvest losses need to be examined.

Knowledge on storage conditions such as the particle size of *C. nutans* for better quality storage is still lacking. Whether powdered or shredded form of *C. nutans* will affect the quality of the product have not been well studied and documented. However, previous studies such as by Stahl-Biskup and Venskutonis (2012) found that dried thyme (*T. vulgaris* L.), in the form of medium or coarsely ground material, is stable and does not loose volatile compounds while finely milled thyme tends to loose volatiles more rapidly. In addition, Renee et al., (2014) showed that significant increase in anthocyanins, carotenoids and ORAC values are recorded as the particle size distribution decreased. Also, the effects of storage duration of *C. nutans* towards the quality of the product need to be studied. There are possible adverse consequences of long-term storage towards the quality of herbs in terms of the bioactive compound. Storage duration could cause destructive reactions such as auto-oxidations or enzymatic splitting and rearrangement of active constituents (Kumar, 2013). Therefore, for sustainable production, and to maintain quality and reduce postharvest losses of this valuable herb, suitable storage form and optimum storage duration practices need to be examined.

In Malaysia, the demand for *C. nutans* tea has increased, however, the tea products have inconsistent quality while guidance for preharvest and postharvest handling is still lacking. As stated by Jin et al. (2016), herbal tea consumption shows regional characteristics associated with local culture, traditions and diversity of local flora. Different products will give different results according to their cultural practices until the postharvest handling and transfer to the consumer. Infusion time also affects the tea quality during preparation at different infusion times. As stated by Komes et al. (2010), the green tea reaches optimum total flavonoid compounds (TFC) at 5 min of infusion and started to decrease after 50 min of infusion. Other results also showed that prolonged infusion time at high temperature could lead to catechin degradation (Cheong et al., 2005).

Due to the increased demand for this herbal tea as natural antioxidants with a broad spectrum action, a variety of *C. nutans* tea is produced and marketed locally. Until recently, analysis of physicochemicals and microbial contamination at different plant harvesting ages (young and mature), are still lacking. To solve the problem of inconsistency in the production of this *C. nutans* tea, a study with the following objectives was conducted:

1. To determine the effects of plant vegetative stages and postharvest drying methods on the physicochemical characteristics and microbial contamination in *C. nutans* dried leaves.
2. To determine the effects of different leaf form (shredded and powdered) and storage duration (0, 4, 8 and 12 weeks) on quality characteristics and microbial contamination of *C. nutans.*
3. To determine the effect of infusion time on the physicochemical characteristics, microbial contamination and heavy metal contents of *C. nutans* tea products found in the Malaysian market.

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